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# Health Effects of Sewage Aerosols Additional Serological Surveys and Search for 'Legionella pneumophila' in Sewage

Illinois Univ. at the Medical Center, Chicago

Prepared for

Health Effects Research Lab.
Cincinnati, OH

Apr 81

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Apr 81

R. Northrop, C. Becker, R. Cordell, M. Sulita, and N Altman.

PERFORMER: Illinois Univ. at the Medical Center, Chicago. School of Public Health.
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Antibody levels to Legionella pneumophila, serogroup 1 and Hepatitis A Virus (HAV) were determined for 433 persons living within a 1.6 km radius of an activated sludge plant. Sera of children 6 to 13 years of age were also tested for antibody to Norwalk Virus. The antibody prevalence rates for L. pneumophila, HAV and Norwalk Virus were 23.2%, 31.55 and 7.1%, respectively. The prevalence of L. pneumophila antibody was higher than expected but not the others. Seroconversions were not observed except for a 4-fold rise in HAV antibody in one pair of sera suggesting new infections did not occur in the 8-month study period by any of these agents. Using an index of exposure for the number of viable organisms generated at the plant to which participants were exposed, the average index for persons with antibody to L. pneumophila, or HAV, was not different from the averages of those without antibody. In addition, there was no association between how close people lived to the plant, or how long they lived in the study area, and being seropostive. These findings augment b, ....

KEYWORDS: *Activated sludge process, *Viruses, *Sewage treatment effluents.

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April 1981

# HEALTH EFFECTS OF SEWAGE AEROSOLS:

## Additional Serological Surveys and Search for *Legionella pneumophila* In Sewage

by

R. Northrop, C. Becker, R. Cordell, M. Sulita
N. Altman, R. Anderson, and J. Kusek
School of Public Health
University of Illinois Medical Center
Chicago, Illinois 60680

Grant #R-805003

Project Officer

Walter Jakubowski
Epidemiology Division
Health Effects Research Laboratory
Cincinnati, Ohio 45268

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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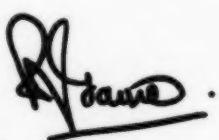
## FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report provides additional assessment and discussion of data obtained in a community having an activated sludge wastewater treatment plant, to determine if the plant could be related to the occurrence of Legionnaire's Disease and Hepatitis A infections in the community and whether the Legionnaire Disease bacterium exists in sewage and aerosols. With a better understanding of the health effects, measures can be developed to reduce exposure to potentially harmful materials.



R. J. Garner
Director
Health Effects Research Laboratory

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## ABSTRACT

Antibody levels to Legionella pneumophila, serogroup 1 and Hepatitis A Virus (HAV) were determined for 433 persons living within a 1.6 km radius of an activated sludge plant. Sera of children 6 to 13 years of age were also tested for antibody to Norwalk Virus. The antibody prevalence rates for L. pneumophila, HAV and Norwalk Virus were 23.2%, 31.5% and 7.1%, respectively. The prevalence of L. pneumophila antibody was higher than expected but not the others. Seroconversions were not observed except for a 4-fold rise in HAV antibody in one pair of sera suggesting new infections did not occur in the 8-month study period by any of these agents.

Using an index of exposure for the number of viable organisms generated at the plant to which participants were exposed, the average index for persons with antibody to L. pneumophila, or HAV, was not different from the averages of those without antibody.

In addition, there was no association between how close people lived to the plant, or how long they lived in the study area, and being seropostive. These findings augment but still did not prove the previous findings that sewage aerosols emitted from this plant had no overt health effects.

In search of L. pneumophila in sewage and aerosols, guinea pigs were inoculated with the particulates of sewage and air samples collected at the plant. The inoculum was adjusted to contain not more than $10^6$ organisms by standard plate count. In one experiment 4 animals were inoculated with undiluted sewage and L. pneumophila was identified in spleen cells by direct fluorescent antibody (DFA) 6-7 days later. After 10-fold dilution of the sample none of the 4 animals had evidence of infection. In a second experiment using undiluted inocula, 2 of 4 guinea pigs developed infection as determined by DFA. Infections were not detected in animals inoculated with the respective aerosol samples. These findings suggested that viable L. pneumophila or a related agent occurs in sewage.


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**ACKNOWLEDGMENTS**

Dr. Kerby Fannin of the Illinois Institute of Technology Research Institute obtained the air samples and sewage grab samples for the Legionella pneumophila search.

Dr. Cecil Lue-Hing, Director of Research, Metropolitan Sanitary District of Greater Chicago arranged for admission onto the North Side Sewage Treatment Works grounds to collect air and sewage samples.

The Center of Disease Control, Atlanta, Georgia provided the serological test reagents for the Legionella pneumophila fluorescent antibody (both direct and indirect) analyses.

We gratefully acknowledge the cooperation of Dr. H. Ehrhard, Director, Division of Laboratories, Illinois Department of Public Health in providing serum samples and for use of the gamma radiation counter is appreciated.

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# SEROLOGICAL SURVEYS FOR *LEGIONELLA PNEUMOPHILA* HEPATITIS A AND NORWALK VIRUSES

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# SECTION 1

# INTRODUCTION

## OBJECTIVES OF STUDY

This study is an addendum to a previous study on the health effects of sewage aerosols carried out in the vicinity of the North Side Sewage Treatment Works (NSSTW) in Skokie, Illinois (1). At the time of the original study, serum samples were collected from 433 persons in the study population. The sera were initially tested for antibody to several enteroviruses in an attempt to discover whether there was a relationship between the level of exposure to sewage aerosols and infection with these enteroviruses.

The purpose of this addendum was to determine whether there was relationship between exposure to sewage aerosols and prevalence of antibodies to Legionella pneumophila (2) and Hepatitis A virus (3) or Norwalk virus (4).

## STUDY DESIGN

An area within a 1.6-kilometer radius of the NSSTW was designated as the study area, which was estimated to consist of approximately 16,000 persons.

The design and conduct of this main study has been described in detail (1). Of the 16,000 possible residents in the study area a stratified random sample of 2,378 (approximately 15%) were recruited for a questionnaire survey. Of these, 318 persons voluntarily gave two blood samples, one at the beginning and a second at the end of the eight month study period and an additional 115 people gave one blood sample either at the beginning or at the end of the study. Sera of these 751 bloods were tested for presence of antibody to Legionella pneumophila or Hepatitis A virus. Blood was not collected from children under six years of age and only blood samples from children six to twelve years of age were tested for antibody to Norwalk virus.

The presence of antibody in the first or only blood collected was used to calculate prevalence rates of immune individuals. A four-fold rise in antibody between paired sera was taken as evidence of recent infection and used to calculate incidence rates of infection.

Rates were then evaluated with respect to the demographic characteristics, income, residence, occupation, underlying health conditions, and exposure index to viable aerosol particles previously documented from the questionnaire, health, or environmental surveys described (1).

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From these data sets a dose-response relationship between different levels of exposure to sewage plant emissions and illness and infection rates was investigated.

Standard techniques such as regression and analysis of variance were used to relate the health and environmental data.

## BACKGROUND AND LITERATURE REVIEW

### Legionella pneumophila

An epidemic of pneumonia at the Legionnaires' convention in Philadelphia in the summer of 1976 led to the discovery by McDade and Shepard of the Legionnaires' Disease Bacterium (5) now called Legionella pneumophila (2). Investigations of this and other epidemics caused by this bacterium have produced evidence implicating an environmental source (6-9). Isolation of the bacterium from the environment during or following the epidemic was possible in a few cases. some serological studies of populations exposed to Legionella pneumophila in these epidemics were done with varying results. It would be useful therefore to study a population of healthy individuals living around an activated sludge plant with a defined environmental exposure to sewage aerosol in a non-epidemic situation. Isolation of Legionella pneumophila was also attempted two years after the sera were collected and will be discussed later.

### Hepatitis A

Although the Hepatitis A virus has not yet been officially classified, evidence indicates that it is most likely an enterovirus of the picorna- or parvovirus type (10). While enteroviruses have been infrequently identified in sewage aerosols to date, they have been found in abundance in sewage (11-14). Primary and trickling filter treatments have only a modest effect on virus levels in sewage. While activated sludge treatment removes 90 to 98% of the enteroviruses there is concern, however, that a large number of potentially infectious virus particles may be aerosolized in the process creating a health hazard for sewage treatment plant workers and persons residing nearby (11). The respirable particles may become trapped in the nasopharynx of exposed persons, an appropriate site for an enterovirus to initiate an infection. Thus, the danger of Hepatitis A virus infection occurring through inhalation of the virus into the nose or mouth is a possibility (12).

Previously, little, if any, work has been done concerning the occurrence of Hepatitis A virus in sewage aerosols. Although a study was done in Israel which suggested a population living near a wastewater irrigation system experienced two to four times the incidence of infectious hepatitis compared to a control population (15), the interpretation of these findings awaits reanalysis (16). It was thus felt that since sera and environmental data were available from the original NSSTW study, a serological screening for antibody prevalence in the study population, as related to exposure indices would be important. Serological analysis would be an appropriate indication of experience with the Hepatitis A virus since most infections are subclinical and are not manifested as overt disease.

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**Norwalk Virus**

**Norwalk virus is one of the viral agents recently shown to be associated with epidemics of gastroenteritis in older children and adults. Infections, either clinical, or subclinical, are relatively infrequent (<< 5%) in children before age 10 years while 50-60% of adults have serological evidence of such infections. The virus is a small, parvovirus-like particle, apparently spread by the fecal-oral route and shed to maximal amounts in feces during episodes of diarrhea.**

**Since the Norwalk virus** occurs in stools, this suggests that it may be **present in sewage and possibly aerosols for spread to exposed persons around sewage treatment works. Sera of children 12 years of age and less in the study population were tested to determine if the prevalence of 5-10% antibody would be found as expected from previous studies (4).**

**Rotaviruses, which are Reovirus-like viruses, have also been associated with epidemics of diarrhea but in this case in infants and children less than 3 years of age. Seroepidemiological studies have shown that over 70% of children in the 3-5 year old age group have Rotavirus antibody. There was initial interest in determining Rotavirus antibody prevalence in the study population aged 12 years and less, but since blood was not collected from children under 6 years of age, it was decided to withhold those determinations until the Norwalk virus antibody data were evaluated.**

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## SECTION 2

## CONCLUSIONS

1. The prevalence of antibody to Legionella pneumophila, serogroup 1, in the study population was higher than expected, 23.2%, but there was no association between total viable particle exposure index, or length of residency in the study area to implicate aerosols from the sewage treatment plant as a source of exposure.

2. New cases (incidence) of Legionella pneumophila, based on a 4-fold antibody rise over the 8-month observation period, were not detected.

3. Prevalence of antibody to Hepatitis A Virus was found to be 31.5% which was an expected rate for that study population. Personal exposure indices or length of residency in the study area were not associated with having antibody.

4. One new infection with Hepatitis A Virus was detected serologically suggesting a two- to three-fold higher incidence rate than expected, but the single case was considered a spurious finding.

5. Norwalk virus antibody in the 6-12 year old age group occurred at an expected prevalence rate (7.1%) suggesting no unusual occurrence of infection by this gastroenteritis virus in the population.

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## SECTION 3

## RECOMMENDATIONS

1. **Prospective studies of populations newly exposed to sewage aerosols would provide more definitive information on the risk of infection by Legionella pneumophila, Hepatitis A or any of the gastroenteritis viruses.**

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SECTION 4

METHODS OF PROCEDURE

STUDY AREA AND POPULATION

The study area was located within a 1.6 km radius of the NSSTW, an activated sludge plant. The estimated population within this area was 15,850 persons or 5,600 households. The socioeconomic distribution was relatively homogeneous with most residents being in the middle or upper middle socioeconomic class.

For sampling purposes, the study area was divided into three concentric zones surrounding the plant. Sampling Zone 1 consisted of the area between 0.0 and 0.8 km from the center of the plant. Sampling Zone 2 was comprised of the area within a 0.8 to 1.2 km radius and Zone 3 was the 1.2 to 1.6 km radius of the plant. A disproportionate stratified random sample formed the basis of selection of households chosen to receive a Health Questionnaire, and a subsample of these was asked to provide blood samples at the beginning and end of the 8 month study period. Four hundred and thirty-three (433) blood samples (318 paired and 115 single specimens) were collected from the study participants in 290 households, distributed throughout the study area.

Methodology for Environmental Monitoring Program

The environmental monitoring program was designed to characterize the nature and degree of exposure of the study population to pollutants emitted during the sewage treatment. Three types of materials were monitored in air and sewage: viable particles, non-viable particles and gases. Measurements were made over an 8-month period at the sewage treatment plant and in the surrounding community.

The environmental monitoring data were used to develop a "personal exposure index" for the residents of the study area. This was accomplished by using maps of concentration isopleths generated from the air pollution data. The "personal exposure index" takes into account meteorological factors as well as the actual measured pollution levels. This index provided a measure of the average exposure of each study participant to viable particles (mainly bacterial) emitted from the plant during the 8 month study period. The index was then integrated with serology data for statistical analysis of a possible correlation between exposure to viable particles and prevalence of antibody to *Legionella pneumophila* and Hepatitis virus.

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## Methods for Serological Analysis

### Legionella pneumophila

The Legionnaires' Disease antibody titers were determined on all sera, single and paired, by the indirect fluorescent antibody (IFA) method developed and described by the Center for Disease Control (CDC) (17). Briefly each serum was diluted serially in phosphate buffered saline (PBS) from an initial 1:16 dilution of the serum in 3% normal chicken yolk sac-PBS suspension. The 1:64 dilution was screened and all positive or questionable positives were repeated at dilutions of 1:64 through 1:512.

The test was performed by adding the serum dilutions to 12-well depression slides bearing Legionnaires' Disease Group I Philadelphia 1 Antigen. The slides were incubated, washed, and dried and antihuman fluorescein-labeled globulin was added. The slides were again incubated, washed, dried and mounted with cover slips. The slides were then examined by fluorescence microscopy. Dilutions showing one-plus or greater fluorescence of 50% or more of the bacilli were taken as positive for antibody. Positive and negative antigens and antisera were included as controls on each slide.

### Hepatitis A

Four hundred and twenty-nine (429) serum samples were tested for antibody to the Hepatitis A virus. Three hundred and eighteen (318) of the samples were paired sera, and 111 were single samples. A period prevalence of Hepatitis A antibody was calculated in order to include cases where one specimen (either the first or second) was missing.

The availability of a large number of paired sera also afforded an opportunity to check for any seroconversions which might have occurred during the study period.

The presence of antibody to Hepatitis A virus (anti-HAV) was determined using the Abbott Laboratories HAVAB kit. The kit was a radioimmunoassay which allowed for a qualitative or quantitative determination of an anti-HAV. For antibody screening purposes, the qualitative assay was deemed preferable. The procedure was carried out according to the instructions provided by Abbott Laboratories, as follows: 10µl of each serum was placed in separate reaction wells, followed by addition of 200 µl of anti-HAV $^{125}I$ (human). One Sephadex bead, coated with inactivated Hepatitis A virus (primate), was placed in each reaction well, covered and allowed to incubate for 24 hours to allow for competitive binding of anti-HAV$^{125}I$ and any anti-HAV which may have been present in the serum. For each run of 100 sera, three negative controls (recalcified human plasma, non-reactive for anti-HAV and $HB_sAg$) and two positive controls (recalcified human plasma, reactive for anti-HAV, non-reactive for $HB_sAg$) were subjected to the recommended procedure. At the end of the 24 hour incubation period, each bead was thoroughly washed with distilled water (25ml) and radioactivity levels were measured in a gamma scintillation counter (Tracor Analytic Model 1285). Evaluation of reactive substance (antibody) in sera was accomplished by determining a cutoff value (in cpms) with which to compare unknown serum cpms.

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This cutoff value was determined, for each run of 100 sera by the following formula:

$$\text{Cutoff Value} = \frac{\text{Negative Control Mean} + \text{Positive Control Mean}}{2}$$

Thus, sera with cpm values greater than the cutoff value were considered to be non-reactive for anti-HAV, while sera with cpm values less than the cutoff value were considered to be reactive for anti-HAV.

Norwalk Virus

Paired sera from fourteen children 6-12 years of age as previously described (1) were sent to Neil R. Blacklow, M.D., Section of Infectious Disease, University of Massachusetts Medical School, Worcester, for the antibody determination. The radioimmuoassay (RIA) method used has been recently described (4).

Statistical Methods

Several variables were examined in order to determine whether they were correlated with seropositivity to either Hepatitis A or Legionella pneumophila. The variables which were analyzed included: age; sex; income; smoking status; presence of chronic respiratory, cardiovascular or gastrointestinal disease and other less frequent forms of chronic conditions; activity; history of clinical Hepatitis A and B; hours spent outside the study area per week; geographic distance of household from plant and viable particle exposure index.

Chi-square analysis was performed on all of the variables except exposure index. Where appropriate, a Mantel-Haenszel test (18) was also performed in order to check for trends which might not have been apparent in the individual Chi-square tables.

All of the variables with the exception of geographic distance from the plant, past history of infectious or serum hepatitis, and activity, were analyzed in relation to seropositivity or negativity with a multiple logistic regression (MLR) technique. The purpose of this analysis was to provide a sensitive measure of the effect of each variable on antibody, presence or absence, while controlling for all other variables.

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## SECTION 5

## RESULTS AND DISCUSSION

### *Legionella pneumophila* serosurvey

The serological data for *Legionella pneumophila* were reported at titers, i.e., reciprocal of the dilution, of <64, 64, 128, 256, and ≥512. Titers of <64 were not considered specific and those of 64 of questionable specificity. Seropositive sera with titers of 128 or greater, were taken as presumptive positive (17,19). It must be pointed out that prevalence data for *Legionella pneumophila* antibody are not readily interpreted because of specificity of the IFA reaction and the duration of antibody *in vivo* are not yet known (19).

Published studies of *Legionella pneumophila* antibody prevalence have yielded varying results. Briefly, studies of hospital and other employee groups exposed to Legionnaires' Disease patients and point source epidemics indicated that 1.8% of these populations have titers ≥128 (19-27). Population studies in epidemic situations suggest that 5.7 - 35.0% of the people have titers (22,23,26). Non-epidemic associated groups however have yielded prevalence of significant antibody in the 0.5-5.0% range (21,23,25-28) and the group with a prevalence of 5.7% was considered as probably indicating endemicity. Thus far, high prevalence of *Legionella pneumophila* antibody has been demonstrated only in association with epidemic or endemic exposures. As stated earlier, it is difficult at this time to interpret how specific a positive titer is for *Legionella pneumophila* infection unless a four-fold rise in antibody is demonstrated. Some reactivity of even very high titer sera can be removed with *Escherichia coli*, indicating non-specific cross reactions (29) may occur.

In this study Legionnaires' Disease antibody titers were distributed as shown in Table 1. There was an overall prevalence of 23.2% for persons with antibody titers of ≥128. New cases (incidence) of *Legionella pneumophila* infection were not found; in the 318 paired sera tested, none revealed a four-fold rise in titer. There was no significant difference in the mean exposure index for persons with titers ≤64 and those with ≥128; the mean indices were 163.8 and 155.6, respectively.

The relationship beteeen antibody prevalence and exposure index was negative using the multiple logistic regression analysis. The antibody titers seem to be of some importance when compared to other serosurveys (27) but they do not appear to be associated with exposure to plant aerosols in this study. Analysis by distance of residence from the plant rather than exposure index (Table 2) was consistent with that interpretation.

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TABLE 1. PREVALENCE OF LEGIONELLA pneumophila ANTIBODY BY TITER

| Reciprocal titer | Sera Reactivity No. | % | Accumulated % Reactivity |
|---|---|---|---|
| <64 | 218 | 50.6 | 76.8 seronegative |
| 64 | 113 | 26.2 | |
| 128 | 69 | 16.0 | |
| 256 | 25 | 5.8 | 23.2 seropositive |
| 512 or | 6 | 1.4 | |
| Total | 431[a] | 100.0 | 100.0 |

[a] 2 sera missing

TABLE 2. DISTRIBUTION OF LEGIONELLA pneumophila SEROPOSITIVE PARTICIPANTS BY DISTANCE OF RESIDENCE FROM PLANT

| Distance Residence from Plant | | Distribution of Antibody Titer <64 No. | % | >128 No. | % |
|---|---|---|---|---|---|
| 0 | -0.8km | 114 | 34.4 | 28 | 28.0 |
| 0.8 | -1.2km | 104 | 31.4 | 40 | 40.0 |
| 1.2 | -1.6km | 113 | 34.2 | 32 | 32.0 |
| Total | | 331[a] | 100.0 | 100 | 100.0 |

[a] 2 sera missing

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There was no significant association between age and presence of antibody (Table 3) or sex and antibody positivity (Table 4). This was in contrast to the known marked age and sex differential found in cases of clinical disease. (20).

Other variables which were examined for association with antibody were smoking and underlying chronic conditions. Neither was associated with significantly increased antibody prevalence to Legionella pneumophila (Tables 5,6).

TABLE 3. DISTRIBUTION OF LEGIONELLA pneumophila SEROPOSITIVE BY AGE

| Age Groups, years | No. tested | Seropositive[b] No. | % |
|---|---|---|---|
| 6 - 9 | 8 | 1 | 12.5 |
| 10 -19 | 58 | 12 | 20.7 |
| 20 -29 | 55 | 19 | 31.5 |
| 30 -39 | 57 | 13 | 22.8 |
| 40 -49 | 65 | 15 | 23.1 |
| 50 -59 | 74 | 15 | 17.9 |
| 60 -69 | 83 | 20 | 24.1 |
| 69 | 21 | 5 | 23.8 |
| Total | 431[a] | 100 | 23.2 |

[a] 2 sera missing
[b] titers ≥128

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TABLE 4. DISTRIBUTION OF LEGIONELLA pneumophila SEROPOSITIVES BY SEX

| Sex | No. tested | Seropositive [b] No. | % |
|---|---|---|---|
| Male | 218 | 57 | 26.1 |
| Female | 213 | 43 | 20.2 |
| Total | 431[a] | 100 | 23.2 |

[a] 2 sera missing
[b] titers ≥ 128

TABLE 5. ASSOCIATION OF LEGIONELLA pneumophila SEROPOSITIVE AND SMOKING

| Smoking history | No. tested | Seropositive [b] No. | % |
|---|---|---|---|
| Non- Smoker | 190 | 49 | 25.8 |
| Previous smoker | 125 | 25 | 20.0 |
| Smoker | 104 | 25 | 24.0 |
| Total | 419[a] | 99 | 23.6 |

[a] 2 sera missing and 12 children under 12 years of age not included
[b] titer ≥ 128

TABLE 6. ASSOCIATION OF LEGIONELLA pneumophila SEROPOSITIVE AND ANY CHRONIC DISEASE

| Chronic Disease | No. tested | Seropositive [b] No. | % |
|---|---|---|---|
| Yes | 256 | 53 | 20.7 |
| No | 175 | 47 | 26.9 |
| Total | 431[a] | 100 | 23.2 |

[a] 2 sera missing
[b] titer ≥ 128

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**Hepatitis A virus serosurvey**

Four hundred and twenty-nine (429) serum samples were tested, with 135 of these being reactive for antibody to the Hepatitis A virus. This period prevalence rate of 31.5% was consistent with the findings of previous seroepidemiological studies which have been done in U.S. populations (3,30,31).

Only one seroconversion was observed during the course of the 8-month study period indicating an incidence rate of 0.3% in this population. Consequently, only the prevalence data were considered in the statistical analysis. There was no statistically significant difference in the number of seropositives between the sexes; males exhibited a 33.3% prevalence rate and females a 31.5% rate.

Both Chi-square and multiple logistic regression analyses indicated that age and income were strongly associated with reactivity for Hepatitis A antibody, age being directly proportional to antibody prevalence and income being inversely related to antibody prevalence (Tables 7,8). Neither chronic disease activity, hours spent outside the study area, smoking status or previous history of infectious hepatitis were related to seropositivity.

A possible correlation between exposure index and Hepatitis A antibody was evaluated by the multiple logistic regression technique. No significant association was found between these 2 variables, however.

Since the average duration of residence in the study area was shown previously to be 11.1 years (1) and the exposure indices were developed only for the 8 month study period, it was of interest to determine whether the same results would be obtained if geographical distance from the plant site were compared with antibody prevalence. Chi-square analysis showed no significant relationship between geographic locations and prevalence of antibody to Hepatitis A (Table 9).

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TABLE 7. DISTRIBUTION OF PEOPLE WITH REACTIVITY TO HEPATITIS A VIRUS BY AGE

| Age group years | No. tested | Reactivity for Anti-HAV[b] No. | % |
|---|---|---|---|
| 0 - 9 | 8 | 0 | 0 |
| 10 - 19 | 58 | 0 | 0 |
| 20 - 29 | 55 | 2 | 3.6 |
| 30 - 39 | 56 | 17 | 30.4 |
| 40 - 49 | 64 | 17 | 26.6 |
| 50 - 59 | 84 | 31 | 36.9 |
| 60 - 69 | 83 | 50 | 60.2 |
| 70 - 79 | 18 | 15 | 83.3 |
| 80 - 89 | 3 | 3 | 100.0 |
| Total | 429[a] | 135 | 31.5 |

[a] 4 missing observations
[b] $X^2 = 108.33$ with 8df $p = 0.0000$

TABLE 8. DISTRIBUTION OF PEOPLE WITH REACTIVITY TO HEPATITIS A VIRUS BY INCOME

| Annual Income | No. tested | Reactive for Anti-HAV No. | % |
|---|---|---|---|
| Less Than $3,000 | 4 | 4 | 100.0 |
| $ 3,000 - 6,999 | 3 | 2 | 66.7 |
| $ 7,000 - 9,999 | 19 | 10 | 52.6 |
| $10,000 - 14,999 | 34 | 18 | 52.9 |
| $15,000 - 24,999 | 130 | 32 | 24.6 |
| 25,000 | 164 | 44 | 26.8 |
| Don't know | 12 | 5 | 41.7 |
| Refused | 43 | 16 | 37.2 |
| Total | 409[a] | 131 | 32.0 |

a 24 missing observations
b $X^2 = 31.24$ with 7df; $p=0.0010$
$X^2 = 26.33$ with 5df; $p=0.0010$ (exclusive of don't know and refused)

TABLE 9. DISTRIBUTION OF REACTIVITY TO HEPATITIS A VIRUS BY DISTANCE OF RESIDENCE FROM PLANT

| Distance of Residence from Plant | No. tested | Persons with Reactivity for Anti-HAV No. | % |
|---|---|---|---|
| 0 - 0.8km | 142 | 49 | 34.5 |
| 0.8 - 1.2km | 143 | 40 | 28.0 |
| 1.2 - 1.6km | 144 | 46 | 31.9 |
| Total | 429 | 135 | 31.5 |

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In conclusion, the data obtained and analyzed in this portion of the study suggested that aerosols emitted from the NSSTW do not present an obvious source of exposure to the study population with Hepatitis A virus.

Norwalk Virus Serosurvey

Fourteen paired sera were tested for antibody to Norwalk virus. The sera were from those children 12 years of age and less that had submitted to blood samples during the serosurvey as previously described (1). Dr. Neil Blacklow kindly performed the serological RIA on these sera. He reported "all were negative by RIA at our routine screening dilution of 1:50, except for one serum pair both of which were positive at 1:50. The one out of 14 children's sera that were positive from children aged 6-13 years is quite consistent with the 5% positivity rate we find for Americans in that age group". He then cited Reference (4) as the basis for this interpretation. In view of these results, no further analysis of association with exposure indices, etc. was conducted. Likewise there was no justification to perform antibody screening for Rotavirus as explained in the Introduction of this Section.

These findings suggest, within the limits possible with a small subset of the study population that there was no suspicion that the children in the study area experienced unusual amounts of infection with Norwalk Virus and thus by implication not with Rotaviruses.

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SEARCH FOR LEGIONELLA pneumophila IN SEWAGE AND AEROSOLS.

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SECTION A

# INTRODUCTION

## OBJECTIVE OF STUDY

This preliminary report is an addendum to the health effects study in Skokie, Illinois (1) to consider the occurrence of LDB in sewage and sewage aerosols. The specific objectives of this portion of the addendum were: 1.) to determine if there were bactericidal substances in sewage which would have precluded the occurrence of LDB; 2.) to evaluate if the reported methodologies for recovering LDB from environmental samples (32,33) could be applied to sewage; and 3.) to attempt to isolate and identify LDB in sewage and aerosols from the North Side Sewage Treatment Works (NSSTW) in Skokie, Illinois.

## STUDY DESIGN

Grab samples of sewage influent in the aeration basin of the NSSTW were obtained and upwind and downwind air samples were collected during the time the influent traveled through the basin. Following suitable preparation in the laboratory, the particulates of sewage and air samples were inoculated intra-peritoneally into separate groups of guinea pigs. It was necessary to determine whether or not guinea pigs would survive the insult of resident organisms in these samples and subsequently display evidence of infection by the slow growing Legionella pneumophila. Evidence of infection would be based on rise in rectal temperature and/or death and demonstration of Legionella pneumophila antigen in peritoneal cells and splenic tissue by direct fluorescent antibody techniques. Preliminary and parallel experiments were conducted to address the other objectives described above and to facilitate interpretation of the results.

## BACKGROUND AND LITERATURE REVIEW

The Legionnaires' Disease Bacterium (LDB, Legionella pneumophila) has been found in nonepidemic-related habitats such as lakes, streams and soils (32-35). It has also been isolated from residual water in cooling towers of air conditioning units in association with disease outbreaks (33,36), suggesting that disease in man results from exposure to aerosols containing the agent. A number of studies have been recently conducted to investigate whether or not aerosols generated during sewage treatment have adverse health effects. (37).

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## SECTION B

## CONCLUSIONS

1. In two experiments, pathogenic Legionella pneumophila was tentatively recovered from grab samples of sewage entering the aeration basin of an activated sludge plant.

2. A liquid medium containing the particulates captured from approximately 200 $M^3$ of air collected 50 meters upwind or downwind from the sewage aeration basins did not contain viable, pathogenic Legionella pneumophila when inoculated into guinea pigs.

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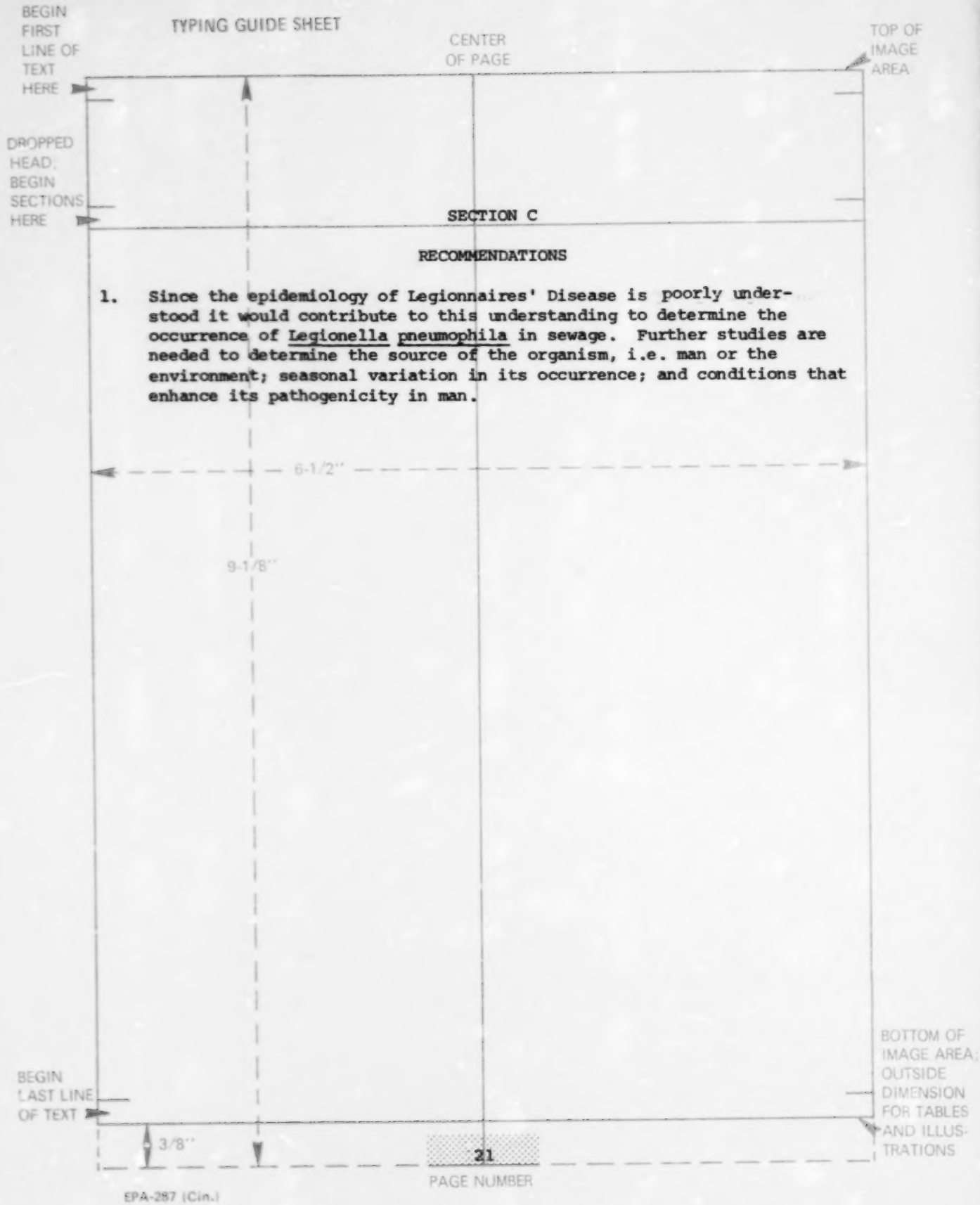
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SECTION C
RECOMMENDATIONS
1. Since the epidemiology of Legionnaires' Disease is poorly understood it would contribute to this understanding to determine the occurrence of Legionella pneumophila in sewage. Further studies are needed to determine the source of the organism, i.e. man or the environment; seasonal variation in its occurrence; and conditions that enhance its pathogenicity in man.
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## SECTION D

## METHODS OF PROCEDURE

### STUDY SITE

Briefly, the NSSTW is an activated sludge plant employing diffuse aeration with a small amount of tapered aeration. The influent is combined sewage and storm water runoff with little industrial waste. Chloriantion occurs after the final settling process. The maximum capacity of the plant is $1.51 \times 10^9$ liters of raw sewage per day with an estimated surface area of the aeration basins of about $5.5 \times 10^4$ $m^2$.

### METHODOLOGY FOR SEWAGE AND AEROSOL SAMPLES

#### Sample Collection

Both sewage and aerosol samples were collected by Dr. Kerby Fannin and staff of the Life Sciences Research Section of the Illinois Institute of Technology Research Institute on September 17 and October 1, 1979. Aerosol samples were collected at upwind and downwind sites within 50 km of the aeration tanks with the precise location dependent on wind direction, accessability and presence of physical obstructions. Air samples were collected using two calibrated, sterilized large-volume air scrubbers at each sampling site. Sterile, antibiotic-free, quarter strength trypticase-soy broth was used as a trapping fluid. Air sampling periods of approximately two hours duration at rates of 0.72-0.84 $m^3$/minute resulted in particle collection equivalent to air volume of 180-193 $m^3$ per sample. Sewage was collected as a single grab sample from one tank inlet at the beginning of the air sampling period and was considered to be representative of the sewage being aerated during the air sampling period.

#### Sample Processing

Samples of sewage were centrifuged in sterile tubes at 2,900 x g for 30 minutes at 5°C (Beckman J-21 Centrifuge with a JA-14 rotor). Supernatant fluids were decanted and the pellets suspended in the original volume with phosphate buffered saline (PBS). Aerosol samples were processed similarly except that pellets were suspended to a final volume of 5.0 ml in PBS. Cultures for total viable particle counts were set up immediately upon receipt in the laboratory and the remainder of the samples were stored overnight at 4°C prior to guinea pig inoculation.

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Preparation of Sewage Supernatant Fluids

Skaliy and McEachern (38) have shown that L. pneumophila survived for up to 415 days in tap water but was inactivated within 24-48 hours by certain bactericidal substances in cooling tower water. In order to determine the presence of bactericidal substances in the liquid fraction of sewage, a sample of sewage collected from the inlet of the aeration tank basins, was transported to the laboratory in wet ice. An aliquot was centifuged at 2,900 x g for 30 minutes at 3°C and filtered through a sterile 0.45 µm pore size membrane (Millipore Corp.). Agar-adapted LDB, grown on charcoal yeast-extract (CYE) agar, was suspended in 5.0 ml of sterile PBS, and further diluted 1:20 in PBS as a working solution. This working solution was used to make serial ten-fold dilutions of the stock L. pneumophila in each of the following diluents: 1.) sterile PBS, 2.) undiluted sewage filtrate, and 3.) sewage filtrates diluted 1:200 in sterile PBS. After the five hour incubation at room temperature, 0.1 ml of each respective dilution was inoculated onto each of three CYE plates using the spread plate technique. Plates were incubated at 35°C in a humidified chamber with 2.5% $CO^2$ and air. Colonies were counted after 6 days.

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Total Viable Particle Counts

Serial ten-fold dilutions of sewage and aerosol samples were made in sterile PBS and 0.1 ml of each dilution was plated onto tryptic soy agar plates using the spread plate technique. Generally, three plates were made for each dilution but, on occasion, only two plates were used. Plates were incubated at 37° under normal atmospheric conditions and colonies counted after 48 hours. The number of colony-forming units (CFU) was calculated per ml of sewage or per $M^3$ of air sampled. Stock L. pneumophila cultures grown on CYE, in agreement with McDade and Shepard (39), were found to be insufficiently pathogenic to cause death in guinea pigs when inoculated with $10^8$ CFU. For that reason, egg yolk-sac material (Philadelphia 1 strain) obtained from the Center for Disease Control was passed once in embryonated hen eggs according to the method of Dade (39); this stock was used in all experiments reported here.

Methodology for Search for Legionella pneumophila

Guinea Pig Inoculations: Adult, male Hartley strain guinea pigs were used in these experiments, except a few females were noted at autopsy. Animals were housed two per cage and kept in an isolation hood with negative pressure air flow. Both animals in each cage received the same inoculum at the same time. Generally, four animals were inoculated with aliquots of a given sample except for the back-titration of the stock, L. pneumophila, in which case two animals were inoculated. The volumes of all inocula were 1.0 ml and all were administered intraperitoneally.

An electronic thermometer (YSI Model 46TUC telethermometer with a LN9631-402 small animal rectal probe) was used to measure rectal temperatures of all animals each day for at least five days pre-and seven days post-inoculation. A baseline preinoculation temperature equivalent to the five day mean was determined for each animal. Animals having temperature increases of more than 0.5°C above the baseline for two consecutive days were considered febrile. Dead animals were stored at either 4°C or -20°C prior to autopsy. Animals

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surviving seven days postinoculation were euthanized with chloroform and autopsied as soon as possible. Peritoneal exudates were collected when present or the peritoneal cavity was washed with 2-3 ml of sterile PBS. Spleens were also collected. These specimens were used for the identification of LDB in guinea pig tissues using the direct fluorescent antibody (DFA) method with reagents obtained from the Center for Disease Control. The same samples were plated onto CYE agar plates and incubated at 35°C in a 2.5% $CO_2$ atmosphere. Plates were examined daily for Legionella-like colonies. The presence of contaminating organism as a result of either poor technique or an occasional ruptured stomach rendered interpretation of most of these plates impractical.

Fluorescent Microscopy: Slides for DFA were fixed in cold acetone for 15 minutes and stained with FITC-labelled rabbit anti-L.pneumophila type 1 (from the Center for Disease Control), for 30 minutes in a humidified chamber. Slides were rinsed under tap water and washed in PBS for 5 minutes prior to mounting and examination. FITC-labelled normal rabbit globulin was used as a control. Slides were examined by the reflected light technique (Leitz Orthoplan with Ploempak 2. la2.2 illumination) and evaluated for the presence of fluorescing bacilli.

## SECTION E

## RESULTS AND DISCUSSION

The first objective of this study was to determine if LDB would remain viable in sewage as the presence of bactericidal agents in sewage would, obviously, render the remainder of the study objectives meaningless. The normal transit time for sewage in the aeration tanks at NSSTW is approximately five hours. Incubation of Legionella in the soluble portion of sewage should give an approximation of the effect of inhibitory agents on LDB under the assumption that any toxic substance would be soluble rather than particulate. Sewage fluids were processed by a combination of centrifugation and filtration in order to exclude any resident microorganisms which would interfere with a quantitative interpretation of the results. A $10^{-2}$ dilution of sewage filtrate in PBS was included in this experiment to determine a dose-response effect of any bactericidal substances and to dilute out any resident microbes not removed by the centrifugation-filtration process. After five hours incubation of L. pneumophila in undilute sewage filtrate, in $10^{-2}$ sewage filtrate and PBS, the number of colony-forming units per ml were $2.79 \times 10^8$, $2.51 \times 10^8$ and $2.12 \times 10^8$, respectively. These results suggested that any inactivation of Legionella caused by substances in the liquid phase of this particular sewage sample was no greater than that occurring in PBS. It is emphasized that the composition of sewage samples might well vary with time of collection, i.e., seasonally, and alter these results.

The isolation of LDB by direct inoculation of sewage onto agar was not feasible due to the lack of a suitable selective medium that would suppress the large quantity and variety of microorganisms normally found in sewage. It was important then, to determine if guinea pigs would act as selective hosts and overcome up to $10^6$ non-Legionella bacteria while supporting infection by L. pneumophila.

The total aerobic bacteria counts from sewage samples collected on September 17 and October 1, 1979 were $1.7 \times 10^6$ and $1.6 \times 10^7$ CFU/ml, respectively. These values were within the previously reported range from this site (1) and suggested that undiluted or 1:10 dilutions of sewage could be tolerated by guinea pigs.

As previously discussed, L. pneumophila grown in yolk sac was known to be infectious for guinea pigs and was added to samples as experimental positive controls. It was already known that the liquid fraction from at least one sewage sample was non-toxic for LDB in vitro. It was important to demonstrate next that sewage solids would not interfere with LDB infection of guinea pigs. Comparative titrations of the yolk sac suspension on agar and in guinea pigs are given in Tables 10 and 11. The stock suspension had a titer

TABLE 10. TITRATION OF L. pneumophila YOLK-SAC MATERIAL ON CHARCOAL YEAST-EXTRACT AGAR

| Dilution of Stock LDB | No. Colonies/Plate Replicate Plates | Average |
|---|---|---|
| $10^{-2}$ | TNTC, TNTC, TNTC | - |
| $10^{-3}$ | 195 171 243 | 203 |

TABLE 11. TITRATION OF L. pneumophila YOLK-SAC MATERIAL IN GUINEA PIGS

| Dilution of Yolk-Sac LDB | No. Animals Inoculated | Animals | | Cumulative No. and % | | |
|---|---|---|---|---|---|---|
| | | No. Surviving | No. Dying | Total Alive | Total Dead | % Dead |
| $10^{0}$ | 4 | 0 | 4 | 0 | 8 | 100 |
| $10^{-1}$ | 4 | 1 | 3 | 1 | 4 | 80 |
| $10^{-2}$ | 4 | 3 | 1 | 4 | 1 | 20 |
| $10^{-3}$ | 4 | 4 | 0 | 8 | 0 | 0 |

Calculation: $\bar{}LD_{50} = \frac{80-50}{80-20} = \frac{30}{60} = 0.5$

$= 10^{-1.5}$ /0.5 ml stock

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of 2.03 X $10^6$ CFU/ml as determined on CYE. Interestingly, a titration carried out on another vial of this suspension four months later had an identical titer of 2.03 X $10^6$ CFU/ml. A titration of this same stock in guinea pigs indicated that there were 31.6 $GPLD_{50}$/ml. This stock suspension had approximately 6.4 X $10^4$ CFU/$GPLD_{50}$ which was similar to the value reported by McDade and Shepard (40).

Guinea pigs inoculated with lethal amounts of L. pneumophila developed a fever which usually reached a maximum of over 40.5°C beginning 3-4 days thereafter. Apparent clinical signs included ruffled fur and discharge from the eyes. Nasal discharge was present but not marked and appeared to be the most common sign of respiratory involvement; there was little coughing or sneezing. A few animals, especially those in the second inoculation experiment developed fevers and outward symptoms but survived the seven day observation period. Autopsies of infected animals yielded varying amounts of peritoneal exudate which when present, appeared as a red mucoid exudate of the peritoneum and viscera. DFA testing of positive control animals revealed fluorescing rods in both splenic and exudate material.

Sewage samples were tested for the presence of Legionella by inoculating guinea pigs with undiluted suspensions of sewage solids (see Methods of Procedure). As positive controls, guinea pigs were inoculated with undiluted and diluted suspensions of sewage solids seeded with 1 $GPLD_{50}$ of stock L. pneumophila. A back titration of L. pneumophila as previously described, was included. As stated in Methods of Procedure, attempts to recover LDB from guinea pig tissues and fluids were unsuccessful. Alternatively, DFA using type specific antisera, was used to screen peritoneal fluids and splenic material for the presence of fluorescing bacilli. All animals receiving the stock L. pneumophila at a dilution of $10^{-2}$ developed fever and died within the seven day observation period. This suggested that neither materials nor microorganisms present in the solid phase of sewage had a marked inhibitory effect on Legionella infection. Apparently, the procedure described by Morris et. al. (32,33) worked as well with sewage as it did with their environmental samples.

Grab samples of sewage were collected and processed and diluted portions of the resuspended sewage particulates were inoculated into four guinea pigs on the assumption that the number of total viable organisms did not exceed the tolerable dose of $10^6$ - $10^7$ organisms. If the undiluted sewage resulted in deaths from non-Legionella infection, it was anticipated that the sample diluted 1:10 would not cause such deaths and might allow evaluation of the presence of LDB. As can be seen in Table 12, L. pneumophila may, in fact, be present in sewage. Of the four guinea pigs inoculated with undiluted sewage, all developed fever, three died 4-6 days postinoculation and all had evidence of LDB antigen by DFA in splenic tissue. Of four animals inoculated with a 1:10 dilution of sewage, two developed fever, none died and one demonstrated DFA-positive LDB antigen in splenic tissue.

The effect of sewage supernatant on LDB viability was also evaluated in guinea pigs. Sewage samples were centrifuged and aliquots of the supernatant

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TABLE 12. RECOVERY OF L. PNEUMOPHILA FROM SEWAGE PARTICULATES AND FILTRATES IN GUINEA PIGS

| | | SIGN OF + INFECTION | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Experiment 1. | | | Experiment 2. | | | |
| Inoculum | Animal No. | Fever | DFA | Death | Animal No. | Fever | DFA | Death |
| Sewage Particulates | H45 | + | + | + | H65A | - | - | - |
| | H46 | + | + | + | H65B | + | - | - |
| | H47A | + | + | - | H66A | a | - | + |
| | H47B | + | + | + | H66B | - | - | - |
| Sewage Particulate +1 $GPLD_{50}$ L. pneumophila | H48A | + | + | + | H67A | - | - | - |
| | H48B | + | + | + | H67B | - | - | - |
| | H49A | + | + | + | H68A | + | - | + |
| | H49B | + | + | + | H68B | + | + | + |
| Sewage Particulate (10-1) | H50A | + | - | - | H69A | - | + | - |
| | H50B | + | - | - | H69B | + | - | - |
| | H51A | - | - | - | H70A | + | + | - |
| | H51B | - | - | - | H70B | - | - | - |
| Sewage Particulate (10-1)+1 $LD_{50}$ L. pneumophila | H52A | + | + | + | H71A | + | - | - |
| | H52B | + | + | + | H71B | + | + | + |
| | H53A | + | + | + | H72A | + | + | + |
| | H53B | + | + | + | H72B | + | + | + |
| Sewage Filtrate | H54A | - | - | - | | | | |
| | H54B | - | - | - | | | | |
| | H55A | - | - | - | | Not Done | | |
| | H55B | + | - | - | | | | |
| Sewage Filtrate +1 $LD_{50}$ L. pneumophila | H56A | + | + | + | | | | |
| | H56B | + | + | + | | | | |
| | H58A | + | + | + | | Not Done | | |
| | H58B | + | + | + | | | | |
| L. pneumophila Stock Back Titration | $10^{-1}$ dil | 2/2[b] | 2/2 | 2/2 | $10^{-1}$ dil | 0/2 | 1/2 | 0/2 |
| | $10^{-2}$ dil | 2/2 | 2/2 | 2/2 | $10^{-2}$ dil | 1/2 | 1/2 | 0/2 |
| | $10^{-3}$ dil | 2/2 | 1/2 | 0/2 | $10^{-3}$ dil | 2/2 | 0/2 | 0/2 |

a Animal died less than two days after inoculation

b No. animals positive /No. animals inoculated

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were inoculated into guinea pigs with and without the addition of one $LD_{50}$. Of the four guinea pigs inoculated with supernatant, only one developed fever, none died and none had evidence of L. pneumophila antigen in tissue. All four guinea pigs receiving the seeded supernatant developed fever, died and contained LDB antigen in spleen impressions. These findings suggested that the aqueous phase of sewage alone contained an insufficient number of viable organisms, including Legionella to cause infection within seven days postinoculation. In addition, there was no evidence that the supernatant fluid had an inhibitory effect on an induced infection.

In summary, the above experiments suggest that 1.) L. pneumophila is present in limited amounts in the particulate portion but not the supernatant portion of sewage; 2.) the soluble portion of sewage did not inhibit growth of or prevent infection by L. pneumophila naturally occurring or seeded in sewage; and 3.) guinea pigs can tolerate $10^6$ - $10^7$ organisms found in sewage and are a useful host in searching of LDB in sewage. This set of experiments was repeated in an attempt to reproduce these findings. A grab sample was obtained from the NSSTW on October 1, 1979 and processed as for the first experiment. A back titration of the stock L. pneumophila yolk sac material was less successful in that guinea pig death did not occur. A similar back titration of this same stock approximately 4 months after this experiment was equally unsuccessful. These results were unexpected in that there was no change in the titration of CFU/ml of the stock LDB on CYE agar over the same time interval. The particulate nature of the yolk-sac material may account for differences in infectiousness of the inocula. The data in the second experiment were somewhat different from the first indicating the need for future experiments.

Air samples were collected adjacent to the aeration tanks to address the health effects question. Furthermore, at the onset of this study there was no assurance that guinea pigs could survive inoculation with sewage hence aerosol samples were collected on the assumption that they would contain fewer non-LDB agents per inoculum. Differences in sampling rate, air volume sampled, wind speed, temperature or relative humidity were not marked on the two dates (Table 13). The quantities of bacteria present in the air samples are shown in Table 14. The samples were concentrated to a final volume of 5.0 ml of which 1.0 ml was used for each guinea pig inoculated. It should be pointed out, as shown in Table 14 for the October 1 sample, that almost 50% of the total viable count was lost during the concentration step. The aerosol experiments were conducted simultaneously with the above described sewage studies. Aliquots of upwind samples and of downwind samples were inoculated into four guinea pigs after processing as described in Methods of Procedure. The total viable particle counts in these samples were well below the $10^6$ CFU limit. Positive controls were not included in order to conserve the aerosol sample for animal inoculation.

There were no differences in the effect of inoculating animals with upwind and downwind samples which would suggest infection of guinea pigs by LDB. Three of eight animals inoculated with upwind samples in both experiments developed fever. None of the animals died and there was no evidence of LDB antigen in splenic tissue postmortem.

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TABLE 13. FIELD SAMPLING AND METEOROLOGICAL CONDITIONS AT NORTH SIDE WASTEWATER TREATMENT PLANT ON SEPTEMBER 17 AND OCTOBER 1, 1979

| Date | Site | Sampler Blower No. | Sampling Rate ($m^3$/min) | Sampling Time | Air Vol. per Sample ($m^3$) | Wind Direct. | Wind Vel. (mph) | Temp. (°C) | R.H. (%) |
|---|---|---|---|---|---|---|---|---|---|
| 9/17 | Downwind(A) | 4/2 | 0.78 | 1127-1330 | 95.9 | SSW | 5-10 | 24-26 | 40-45 |
| 9/17 | Downwind(B) | 11/4 | 0.84 | 1126-1327 | 101.6 | SSW | 5-10 | 24-26 | 40-45 |
| 9/17 | Upwind(A) | 17/3 | 0.84 | 1136-1339[a] | 100.8 | SSW | 5-10 | 24-26 | 40-45 |
| 9/17 | Upwind(B) | 1/1 | 0.72 | 1135-1335 | 86.4 | SSW | 5-10 | 24-26 | 40-45 |
| 10/1 | Downwind(A) | 4/1 | 0.78 | 1139-1347[b] | 98.3 | SE | 5-7 | 22 | 46-57 |
| 10/1 | Downwind(B) | 17/2 | 0.78 | 1152-1352 | 93.6 | SE | 5-7 | 22 | 46-57 |
| 10/1 | Upwind(A) | 1/4 | 0.72 | 1205-1405 | 86.4 | SE | 5-7 | 22 | 46-57 |
| 10/1 | Upwind(B) | 11/3 | 0.78 | 1206-1406 | 93.6 | SE | 5-7 | 22 | 46-57 |

a Sampler down for 3min (1205-1208) due to technical problem.

b Sampler down for 2min (1205-1207) removed bee from sampler.

TABLE 14. TOTAL BACTERIA COUNTS OF AEROSOL AND SEWAGE SAMPLES FROM THE NORTH SIDE WASTEWATER TREATMENT PLANT ON SEPTEMBER 17 AND OCTOBER 1, 1979

| | September 17. | | | October 1. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Before Processing | | | After Processing | |
| Dilution | Sewage | Aerosol (Upwind) | Aerosol (Downwind) | Sewage | Aerosol (Upwind) | Aerosol (Downwind) | Aerosol (Upwind) | Aerosol (Downwind) |
| | ND[a] | | | ND | 3 | CGS[b] | CGS | CGS |
| $10^{o}$ | ND | ND | ND | ND | 0 | CGS | CGS | CGS |
| | ND | ND | ND | ND | 1 | 44 | CGS | TNTC |
| | ND | | | ND | 0 | 3 | CGS | 121 |
| $10^{-1}$ | ND | ND | ND | ND | 0 | 1 | 0 | 141 |
| | ND | ND | ND | ND | 0 | 1 | 0 | CGS |
| | TNTC[c] | | | ND | ND | ND | 0 | CGS |
| $10^{-2}$ | TNTC | ND | ND | ND | ND | ND | 0 | 15 |
| | TNTC | ND | ND | ND | ND | ND | 1 | 0 |
| | 132 | | | TNTC | ND | ND | 1 | 7 |
| $10^{-3}$ | 193 | 6 | 3 | TNTC | ND | ND | 1 | 4 |
| | 193 | 3 | 0 | TNTC | ND | ND | 0 | 1 |
| | 50 | | | CGS | ND | ND | ND | ND |
| $10^{-4}$ | 40 | 1 | 0 | CGS | ND | ND | ND | ND |
| | 32 | 1 | 1 | CGS | ND | ND | ND | ND |
| | 3 | | | CGS | ND | ND | ND | ND |
| $10^{-5}$ | 2 | ND | ND | 20 | 20 | ND | ND | ND |
| | 3 | ND | ND | 12 | ND | ND | ND | ND |
| $10^{-6}$ | 2 | | | 2 | ND | ND | ND | ND |
| | 3 | ND | ND | 0 | ND | ND | ND | ND |
| | 1 | ND | ND | 0 | ND | ND | ND | ND |

[a] ND : Not Done

[b] CGS : Confluent growth due to spreading of motile organisms

[c] TNTC; Too numerous To Count

There appears to be no evidence of LDB infection in guinea pigs inoculated with these aerosol samples. This may be interpreted to suggest that LDB is not captured in aerosol micelles or that a larger air volume should be sampled in order to demonstrate a single $GPLD_{50}$ of L. pneumophila.

# SUMMARY

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The serological survey for antibody to L. pneumophila in the study population suggested that approximately one of four people (23.2%) had been exposed and infected with this bacterial agent some time in the past. No seroconversions were found in paired blood samples taken 8 months apart indicating that people were not being exposed or infected then. Also there was no evidence that people previously infected lived closer to the sewage treatment plant, that they had exposure to more microorganisms originating from the plant in aerosols, or that they had lived a longer time in the study area than people without L. pneumophila antibody.

The absence of an association between having antibody to L. pneumophila and being exposed to sewage aerosols was consistent with a recent report (41) that sewage plant workers exposed to waste water or aerosols did not have higher prevalence rates (9.4%) of antibody than unexposed workers.

In general the prevalence of L. pneumophila antibody has been found to range from 1-14% for populations not involved in epidemics of legionellosis (7, 21, 25, 27, 34).

In order to judge the prevalence of L. pneumophila antibody in the Chicago Metropolitan area we screened 80 sera that were submitted to the State Department of Health in 1978. These sera were randomly selected from all sera of patients hospitalized for non-respiratory problems, or were pre-marital sera. The prevalence rate was found to be 1.2% suggesting that the higher rate in study population deserves further investigation.

In conjunction with the serological survey to evaluate the risk of acquiring L. pneumophila infections from exposure to sewage aerosols, a search was made for the agent in sewage and aerosols microbiologically.

Grab samples of sewage as it entered the aeration basins of the NSSTW were found to contain $10^5$ to $10^6$ viable organisms per ml using standard procedures. It was also found that guinea pigs would survive $10^6$ to $10^7$ of these organisms after intraperitoneal (ip) inoculation in agreement with a subsequent report by McDade, et.al. (40).

These animals routinely developed fever and malaise 6-12 hours post-inoculation (pi) but by 72 hours pi they clinically recovered; the rectal temperature returned to normal and they resumed normal eating habits. A stock of L. pneumophila grown in yolk sacs of embryonated hen's eggs inoculated ip into guinea pigs did not cause fever until after 72 hours pi and frequently resulted in death in 5-7 days. When L. pneumophila was intentionally mixed with undiluted or 10-fold diluted sewage samples and inoculated ip guinea pigs frequently developed fever on day 1 which was usually sustained till death on day 4-7. The spleen and peritoneal cells of these animals contained L. pneumophila as determined by DFA testing. It was possible then to examine sewage samples for presence of L. pneumophila without extensive manipulation of the sample.

Two experiments were then conducted. In the first, L.pneumophila was identified in spleen cells of 4 guinea pigs inoculated with undiluted sewage, but not when animals received sewage diluted 10-fold. It was also shown that

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the supernatant fluid of the sewage particulates did not affect the viability of L. pneumophila. In the second experiment the 10-fold diluted sewage inoculum, but not the undiluted sample, resulted in infection by L.pneumophila in 2 of 4 guinea pigs. In this experiment the supernatant of the particulate was not tested for inhibitory substances.

If L. pneumophila is present in sewage, the source is unknown. Although diarrhea has been reported to be a clinical component of Legionnaires' Disease it is not known if the gastrointestinal tract is infected.(19) It was also recently reported that LDB antigen can be present in urine (42) but whether it is viable LDB has not been tested. Since the NSSTW receives sewage and storm water, and since L. pneumophila has been frequently found in soils and surface water it may have originated from these latter sources. Regardless of the source, it is likely there could be extensive variation in its presence in sewage.

L. pneumophila was not found in any of the aerosol samples tested. The failure to find it does not allow any interpretation. The volume of air sampled to recover LDB may have been inadequate, or even if present in sewage it may not be captured in bubbles and aerosolized.

The prevalence rate of HAV antibody (HAVAB) throughout the study population who gave a blood sample was 31.5%. This was equivalent to what would be expected in middle class urban population in the United States. The occurrence of antibody increased with age and decreased with increased socioeconomic status. There was no association with sex, history of hepatitis, smoking history, or presence of a chronic health condition. As previously reported each person in the study was assigned an exposure index based on the amount of viable organisms the person's residence was exposed to over the 8 month study period. There was no association between the presence of HAVAB and the level of the exposure index. Since the exposure index was based on environmental air sampling for an 8-month period, yet the average length of residence in the study area was over 11 years the distance of the household from the plant site was also compared with the presence of HAVAB. Again, there was no association between distance of the residence from the plant and HAVAB.

One pair of the 318 paired sera collected from this population was found to have significant rise in HAV antibody during the 8 month interval of blood collection. This was equivalent to an incidence rate of 0.3% which was significantly higher than expected based on number of cases reported by the Center for Disease Control (CDC). The Hepatitis Surveillance Report Number 45 (31) conducted by CDC states an annual incidence rate of 14.2 per 100,000 persons, but these figures were based on clinical cases and not infection rates because serological tests were not yet commercially available during the CDC reporting period. Various authorities believe there may be 2 to 10 infections for every clinical case reported. The one seroconversion out of 318 pair sera tested with an interval of 8 months between blood collections was equivalent to an annual incidence rate of 450 per 100,000 persons compared to theoretical estimates up to 150 per 100,000. Although no seroconversions were expected and one was found, the finding that the prevalence rate of HAV antibody was in the expected range and the lack of association

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between HAV antibody and exposure index of distance of residency from the plant cumulatively suggested that sewage aerosols were not the source of HAV infections in this study population.

In addition to Hepatitis A virus, members of two other groups were suspected of being present in sewage. Norwalk virus and Rotaviruses have been etiologically associated with nonbacterial gastroenteritis (43). Fourteen children 6-12 years of age contributed paired blood samples in this study and serological analysis for antibody to Norwalk virus revealed that one of fourteen children had been infected sometime in the past. A prevalence rate of 7.1% was consistent with what was expected and there was no justification for screening sera from other age groups or testing for Rotavirus antibody. Consequently, there was no suggestion of an association between Norwalk virus, or Rotavirus, infections and exposure to sewage aerosols.

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